that of 18S from the same condition. To examine the effects of treatments, each gene was normalized to its level in the no-treatment control. Statistical analysis was performed using a linear mixed-effect model, followed by Tukey's pairwise comparison.
Results: The combination of TNF-α+injury up-regulated the expression of ADAMTS-4,-5, MMP-3,-9,-13, TIMP-3, iNOS, Caspase-3 and IL-6 (Fig.1, $*p<0.05$ vs. no-treatment control). TNF-α, with or without injury, significantly increased GAG loss (Fig. 2, $*p<0.05$) and decreased chondrocyte biosynthesis (data not shown). However, the catabolic effects of TNF-α and TNF-α+injury were abolished in the presence of dexamethasone: both proteoglycan loss and biosynthesis remained at the levels similar to no-treatment controls (Fig. 2).

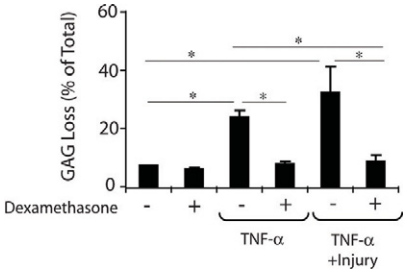

Figure 2

Conclusions: Mechanical injury potentiated the catabolic response induced by TNF-α at the transcript level (data not shown). Short term glucocorticoid treatment effectively abolished the catabolic effects of TNF-α plus injury, preventing both proteoglycan degradation and biosynthesis reduction. Ongoing studies focus on identifying the effects of dexamethasone on expression of inflammatory cytokines and enzymes at the transcript level.

Matrix Biochemistry

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PUTATIVE 3D HUMAN TISSUE MODEL FOR OSTEOARTHRITIS

L. Sun, D.L. Kaplan
Tufts Univ., Medford, MA

Purpose: Osteoarthritis is a major cause of disability during aging. While the mechanisms involved in osteoarthritis are not yet clear, animal models and cell lines are most often used as disease models. However, inherent limitations with these approaches prompt the need for improved options using human tissue systems. A relevant tissue engineering osteoarthritis model *in vitro* would allow more insight into underlying mechanisms and would also provide a useful platform for drug screening.
Methods: Tissue engineered (TE) cartilage was prepared by using silk-derived porous 3D scaffolds seeded with primary human chondrocytes and culturing in chondrogenic medium for 4 weeks. The TE cartilage mimicked cartilage in terms of many aspects of cell and matrix features. An osteoarthritis-like phenotype was induced by culturing the cells with cytokines, a combination of IL-1β, and TNF-α, or culturing the cells with conditioned medium from THP-1 derived macrophages. The later served to generate a broad spectrum of cytokines to represent the infiltration of macrophages *in vivo*. The systems were studied for up to 5 weeks in a static culture.
Results: After 1 week with cytokines induction, matrix anabolism-related genes such as ACAN (GAG) and Col II were dramatically down-regulated; while at the same time genes for matrix catabolism-related enzymes, such as MMP13, ADAMTS4, were up-regulated. As a marker of aging and calcification in osteoarthritis, the Col X gene was up-regulated 7-fold when compared to the untreated (control) group. The data indicated that chondrocytes responded to the cytokine stimulation in these 3D cultures and matrix turnover was initiated, in as a mimic of the disease condition. After 3 weeks, chondrocyte apoptosis was found in the TE cartilage, with a higher or dose response effect when the TE cartilage system was treated with a higher dose of cytokines. Collagen type X expression was confirmed by immunohistology. For TE cartilage treated with conditioned medium from the macrophages, the results and trends were similar to those with the cytokine treated group, except for some differences in magnitude of response in matrix-related gene expression. This difference may due to the complex components present in this conditioned media besides IL-1β and TNF-α.
Conclusions: Silk porous scaffolds provided a compatible environment for three-dimensional tissue engineered cartilage. In the presence of cytokines or conditioned medium from macrophages, TE cartilage showed osteoarthritis-like phenotypes, with results generally consistent with previous clinical studies. This approach may serve as a suitable starting point to generate relevant and useful *in vitro* osteoarthritis models.

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ULTRASTRUCTURAL ANALYSIS OF COLLAGEN IN THE ARTHROPATHY OF ALKAPTONURIA IN VIVO AND IN VITRO

A.M. Taylor, W.D. Fraser, P.J. Wilson, L.R. Ranganath, J.A. Gallagher
Univ. of Liverpool, Liverpool, United Kingdom

Purpose: Alkaptonuria (AKU) is a rare autosomal recessive condition resulting from lack of homogentisate 1,2 dioxygenase (HGD), the enzyme responsible for the breakdown of homogentisic acid (HGA). HGA accumulates in body tissues resulting in ochronosis, the deposition of pigmented HGA polymers that have high affinity for collagenous tissues, primarily articular cartilage of the weight bearing joints. Over time, pigmentation leads to severe joint degeneration presenting as osteoarthritis. The aim of this study was to use light and transmission electron microscopy (TEM) to identify the location of ochronotic pigment in joint tissues in vivo and in an in vitro model.
Methods: Tissues were collected at the time of surgery from AKU patients undergoing joint surgery. C20-A4 transformed human chondrocytes and SaOS-2 osteosarcoma cells were cultured in DMEM in the presence or absence of 0.33×10^{-4} M HGA. All samples were processed routinely for histology and TEM. Paraffin sections were stained with either H&E or nuclear fast red and Schmorl's reagent to highlight pigment. TEM samples were post stained using uranyl acetate and lead citrate.
Results: Macroscopic examination of AKU tissues revealed intense pigmentation of articular cartilage. Synovial and capsular tissues had pigmented and non-pigmented areas. Bone was almost devoid of pigmentation. H&E staining of surgical tissues demonstrated pigment intra- and extracellularly. Chondrocytes, fibroblasts and osteocytes all contained pigment. Ochronotic pigment was located among collagen fibres in articular cartilage, capsule and synovium. Pigment was absent in mineralised collagen fibres of bone.
Schmorl's reagent demonstrated smaller amounts of pigment attached to the collagen fibres, undetected using H&E. Schmorl's staining of in vitro cultures revealed pigment deposits both intra- and extracellularly. Ultrastructural examination of tissue samples revealed pigment deposition on collagen fibres in cartilage and capsule; in some regions pigment was easily identifiable bound to collagen, whereas others had no pigment. Ultrastructural distribution differed between capsule and cartilage. Pigment in capsule was associated with the collagen fibril periodicity and was also